

Invitro Testing of Nanopesticides against *Colletotrichum falcatum* Causing Red Rot of Sugarcane

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Nanotechnology offers numerous advantages and innovative strategies for managing and controlling phytopathogens in agriculture. The use of nanotools helps mitigate the harmful effects and losses associated with traditional fungicides. The development of novel structures and potent nanoformulations ensures more efficient and targeted distribution of growth regulators, nutrients, and other effective pesticides. Furthermore, by enhancing the solubility and prolonging the delivery duration of antifungal compounds, nanostructures designed to combat fungal phytopathogens provide a sustainable and environmentally friendly approach. This method also reduces the toxicity of active substances, making it safer for both crops and the environment. Sugarcane (*Saccharum officinarum*) is a significant cash crop cultivated in tropical and subtropical regions. It is susceptible to numerous diseases, including red rot, whip smut, sugarcane mosaic virus, and red stripe. Among these, red rot caused by *Colletotrichum falcatum* is the most destructive, severely hindering the plant's productivity and leading to a noticeable 5–10% decrease in yield. Various management techniques, such as chemical, cultural, and biological controls, can be employed to manage this disease effectively. The current study evaluated the efficacy of nanoformulated carbendazim and mancozeb against *C. falcatum* under laboratory conditions. The methodology included several steps: collection of disease samples, processing of diseased samples, purification and multiplication of *C. falcatum*, confirmation of *C. falcatum*, in-vitro evaluation of green synthesized nanopesticides against *Colletotrichum falcatum*, and in-vitro evaluation of nanoparticles of commercially available fungicides against *Colletotrichum falcatum*. The results showed that the combination of Carbendazim nanoparticles (NPs) with Silver Nitrate (AgNO₃) and *Azadirachta indica* (neem) exhibited the most effective control, resulting in minimal fungal growth of 16.519 mm. In contrast, the combination of Mancozeb NPs with Zinc Oxide (ZnO) and *A. indica* showed maximum fungal growth of 27.619 mm. The monodispersed spherical morphology of AgNPs and ZnNPs was confirmed through ultrastructure imaging using scanning electron microscopy (SEM) with energy-dispersive X-ray (EDX) analysis. These findings highlight the potential of nanotechnology in developing advanced, sustainable, and environmentally friendly strategies for managing phytopathogens in agriculture. The superior performance of the Carbendazim NPs + AgNO₃ + *A. indica* combination demonstrates the promise of integrating nanotechnology with traditional agricultural practices to enhance crop protection and yield.

Keywords: Nanotechnology, Phytopathogens, Sugarcane, carbohydrates, *Colletotrichum falcatum*, nanoparticles, scanning electron microscopy (SEM), EDX analysis.

INTRODUCTION

A significant cash crop grown in tropical and sub-tropical regions of the world between equator latitudes 36°N and 31°S is sugarcane (*Saccharum officinarum*) (Ram *et al.*, 2022). Its high sugar content in the stem contributes significantly to global sugar production (Menossier *et al.*, 2008). Sugar industry plays important role in Pakistan economy by contributing 3.0 to 4.0 million tonnes sugar, which is 1.9% of

GDP. Average sugar recovery in Pakistan is far below as compared to average world recovery (Shaukat, 2009). Pakistan is ranked fifth in area and eleventh in production among the top producing nations (Khan *et al.*, 2019). Crop requires minimum of 600 mm annual rain fall (Qureshi and Afghan, 2005).

In Pakistan area used under the sugarcane cultivation has increased as compared to other crops (Farooq and Gheewala, 2019). In Pakistan, about 50-60 % of the total cultivation of

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cane is obtained from ratooning. If properly handled, it gives equal amount of yield (Khan *et al.*, 2021). Ratooning decreases the expenses. Approximately 60% of the entire output is crushed in the mills, 30% is used as raw sugar, and 10% is stored for seed. It is also used for chewing in rural areas of the Pakistan (KHAN, 2015). As compared to other crops in Pakistan, the growing area of sugarcane has increased. 50-60 % of the total cane crop is obtained by ratooning in Pakistan (Arshad, 2021).

In Pakistan, there have been recorded cases of red rot, whip smut, sugarcane mosaic virus, and red stripe (Anwar *et al.*, 2010). Fungus causes three major sugarcane diseases that are Red rot of sugarcane, Whip smut of sugarcane and Pokkah Boeng. Red rot among these fungal diseases is one of the most destructive and have extreme threats in many countries under favourable conditions (Sharma *et al.*, 2017). It is registered in 68 developing world countries (Bharti *et al.*, 2012). The disease was first identified in Pakistan in 1986, however it was first reported in Java in 1983 (Abbas *et al.*, 2016). A pathogen *Colletotrichum falcatum* and *Glomerella lucimansensis* are responsible for the disease. The family of *C.falcatum* is *glomerallaceae* (Alexander *et al.*, 2002). It is also called "cancer of sugarcane."

The description of the fungus depends both on its intracellular and intercellular mycelium (Viswanathan, 2021). The asexual fruiting bodies on the host plant's surface are minute, velvety shape (Viswanathan, 2021). Hyphae are thickly walled and tip-pointed. Hyaline, linear, or club-shaped conidiophores produce colorless, single-celled conidia with thin walls (Kumar *et al.*, 2011), under favorable conditions disease is widely spread in susceptible varieties. In Pakistan, it is mainly reported in the central and northern areas of Punjab. About 29–83% of the cane weight and 31–75% of the sugar recovery is reduced because of the fungal attack (Misra *et al.*, 2022). Worldwide, this disease is estimated to have caused 5–10% of losses (Viswanathan and Samiyappan, 2002).

Control of red rot is still a biggest challenge for plant pathologists. For controlling of the disease we must know about the pathogen virulence and time for disease development (Wang *et al.*, 2024). Red rot can be managed by different strategies like chemicals and plant extracts. Diagnosis of the pathogen is primary step in management of any disease. For detection of *colletotrichum falcatum* in sugarcane ELISA, DIBA and Western blot methods are used (Hossain *et al.*, 2020). The use of resistant varieties is the most effective strategy against red rot (Viswanathan *et al.*, 2011). Due to lack of implementation of Quarantine laws pathogen has spread to different regions (Chand *et al.*, 1974). Reduced inoculum by using healthy seed, crop rotation and field sanitation destroy the infected material, crop debris and stubble (Satyavir, 2003). Use long setts for plantation for the control of soil borne inoculums (Anwar *et al.*, 2010). By using various chemicals such as Carbendazim and Benomyl-

infected conditions, some reduction in red rot fully removes sett-borne infection (Agnihotri, 1983).

Different plant extracts are used for controlling the disease which is the cheapest way (Ahmad *et al.*, 2016). That is why, nano-formulation of both chemicals and phyto extracts were evaluated in vitro for management of red rot of sugarcane (Goyal *et al.*, 2023). From time to time, new pathogenic strains of *Colletotrichum falcatum* have been identified due to the collapse of varieties and disruption of the entire varietal structure in some regions of the world. Losses of disease are increased because of the growth of the new strains. The mechanism by which new races evolve in nature is still unclear. These changes may be due to following methods i.e., mutation, hybridization and heterokaryosis (Pasha *et al.*, 2023). Present study was based on evaluation of nanoformulated phytoextracts and chemicals under lab conditions against *colletotrichum falcatum*, causing red rot of sugarcane.

Historically, varieties of chemical pesticides, such as Difenconazole and Carbendazim, have been widely used to treat fungal disease (UL Haq *et al.*, 2020). However, the most popular systemic fungicide is carbendazim, which is renowned for being both safe and effective. Deterioration of the environment and fungal resistance to chemical pesticides are growing constraints resulting from the overuse of these pesticides in agriculture. (Hassan *et al.*, 2010). Scientists are always investigating and assessing the risks associated with pesticide use in the terrestrial environment (PROTECTS project, Ireland). The utilization of nanoparticles (NMs) as agrochemicals is one of the many potential uses of nanotechnology (Duhan *et al.*, 2017; Shang *et al.*, 2019). However, some scientists believe that direct NP released into the environment is harmful to all living things, including plants (Kumari *et al.*, 2011). On the other hand, papers exist that state that NPs applied to plants have no cytotoxic or genetic consequences (khodakovskaya *et al.*, 2009; Lee *et al.*, 2010). It's possible that the roots and leaves of the plants will absorb the NMs via the same pathways as the heavy metals do. Hence, because of their high specificity, superior efficiency, huge surface area to volume ratio, and quick reaction rate, NMs can be used to manage plant diseases in a way that is both economical and environmentally beneficial. Interest in creating and using NM-based products in the food and agricultural industries has grown (Gopal *et al.*, 2013; Peters *et al.*, 2016) But it's important to keep a close eye on the possibility of Ag building up in the food chain. The objectives behind this work were evaluation of nanoparticles of different commercial fungicides against *Colletotrichum falcatum* and evaluation of nanoparticles of different phytoextracts and metals against *Colletotrichum falcatum*.



MATERIALS AND METHODS

Collection of disease samples: The disease mostly appears in the irrigated or water logged regions of the province. Samples of the disease were collected from Ayub Agriculture Research Institute, Faisalabad. These samples were brought into the lab for the purpose of isolating the fungal species and assessing the effectiveness of several nanopesticides against *Colletotrichum falcatum*, which causes red rot of sugarcane.

Media Preparation for the Isolation of fungus: Potato dextrose agar (PDA) medium was prepared for isolation of *Colletotrichum falcatum* with the following ingredients: Distilled water (1 Litter), Glucose (20g), Potato starch (20g), and Agar Agar (20g). In one litter of water peeled potatoes were boiled for 30 minutes. Through cheese cloth boiled water was filtered and poured into the bottle. 20g of agar and 20g of glucose were added to boiled water. Agar agar was added to solidify the media. Mixing should be done and media should be autoclaved for sterilization at 121°C and 15 psi for one hour. Culture was allowed to cool, and poured into Petri plates for isolating the fungus.

Processing of Diseased Samples: Samples (sugarcane stalks and stems) packed in paper bags and brought into the lab to exhibit typical Red Rot disease symptoms. To get rid of the dust and other undesired materials, the samples were cleaned with tap water. The disease samples were cleaned, then cut into small pieces and placed in a laminar flow chamber to be disinfected using 0.5% sodium hypochlorite. Potato Dextrose Agar medium was added to 9x8cm Petri dishes. Four to five disinfected pieces of disease samples were placed on Petri plates with the aid of sterile forceps and covered with tape once the media on the plates had cooled. For 48 hours, plates were kept in the incubator at 28-30 °C.

Purification and multiplication of *C.falcatum*: The growth of the fungus was seen after 48 hours of incubation. A single colony of the fungus was selected and streaked across PDA-containing petri plates using a needle. Plates were inoculated, wrapped, labeled and again placed in the incubator for the next 48 hours. After incubation, the fungus's purified growth was monitored and investigated under a microscope to verify that the culture was pure.

Confirmation of *C.falcatum*: Numerous slides were prepared from the purified pure culture and examined under the microscope to verify the characteristics of the red rot causative organism. Typical characteristics of the spores of the pathogen, including aseptate hyphae with hyaline appearance, were confirmed under the microscope. From these typical characteristics of the spores, it was confirmed that the isolated fungus from these diseased samples was the fungus called *Colletotrichum falcatum*. After confirmation of the red rot pathogen, the multiplied inoculum stored for further use.

In-vitro evaluation of green synthesized nanopesticides against *Colletotrichum falcatum*: The preparation of plant

extracts involved the use of two plants: neem and turmeric. These plants were brought from the nursery, shade-dried, and then oven-dried at 28°C to eliminate moisture (Muhammad *et al.*, 2018). These plants were ground into a fine powder, which was then reconstituted with water to create two concentrations of each plant extract after being passed through muslin cloth (Muhammad *et al.*, 2018). 25g and 50g of the each plant extract were added into 100 ml of distilled water separately to make solutions. This solution was filtered through muslin cloth. After separately combining 45 ml of 1 mM silver nitrate (AgNO₃) with extracts of neem and turmeric, a colour shift was seen that confirmed the formation of AgNPs (Thakur *et al.*, 2022). Freshly prepared 50 ml extracts were placed in a 100 ml beaker and heated to 70–80 °C in order to synthesize ZnONPs (Naseer *et al.*, 2020). Subsequently, the hot pericarp extract was gradually mixed with 4g of zinc nitrate, and a reddish-brown solution produced right away. Using a magnetic stirrer, this reaction mixture was heated at 70-80°C (Naseer *et al.*, 2020). Heating continued until a reddish orange paste formed. As the reaction developed, its color gradually changed from reddish-brown to pale yellow. After moving these pastes to a ceramic crucible, they were heated for two hours at 400°C in a furnace. Then, using a pestle and mortar, these components were ground into a powder. Various concentrations of stock solution have been prepared for application. Using the technique of poisoned food (Singh *et al.*, 2008) the antifungal capacity of phytoextracts was assessed. In a laminar flow chamber, before poured into petri plates each concentration of plant extract was mixed well with PDA. Holes were made in the center of the each petri plate and purified culture of the *C.falcatum* was placed. Plant extracts at each concentration were well combined with PDA and then transferred into petri plates within a laminar flow chamber. At 28-30°C, petri plates were incubated. After 24, 48, and 72 hours, the growth of the fungus was recorded using the following formula (Damasceno *et al.*, 2019).

$$\text{Inhibition of mycelia growth (\%)} = \frac{(C - T/C)}{C} \times 100$$

In-vitro evaluation of nanoparticles of commercially available fungicides against *Colletotrichum falcatum*: The commercially available fungicides Carbendazim and Mancozeb were evaluated against *Colletotrichum falcatum*. Nanoparticles were prepared by adding 0.5g of each fungicide added into 50ml distilled water having 0.016 g silver nitrate in a beaker to make AgNPs of these commercial fungicides (Estrada-Martínez *et al.*, 2021). In another beaker same pouring was done separately in 5 g zinc nitrate to make ZnNPs of commercial fungicides. Both beakers were boiled at 70-80°C. Later, 5ml of NaOH was added slowly in each beaker. The contents of each beaker were transferred to a ceramic crucible followed by heating in a furnace at 400°C for 2 h. These materials were then turned to powder form using pestle and mortar. Stock solution of the nanoparticles was prepared. Different concentrations of the NPs were prepared and added



into 100ml of distilled water. Using poisoned food technique each concentration was evaluated against the pure culture of *C.falcatum* (Ghazanfar *et al.*, 2017). In autoclaved media various concentrations of each nanopesticide was poured into Petri plates. Fungal culture was placed at the centre of the plate and incubated at 28-30 °C for 2-3 days for fungal growth. The data was recorded at various durations and concentrations of the applied nanopesticides.

RESULTS

Invitro evaluation of Nanoformulated phyto-extracts against *Colletotrichum falcatum*: Treatments (T), Concentrations (C), Durations (D) and their interactions including treatments × concentrations (T×C) and treatments × durations (T×D) showed significant results (Table 1). Combination of nanoformulated *Azadirachta indica* and *Curcuma longa* exhibited minimum fungal growth (25.311 mm) followed by *A.indica* NPs (30.009 mm), *A.indica* (32.504 mm), *C.longa* NPs (35.315 mm) and *C.longa* (40.522 mm) as compared to control. The interaction between treatment and concentration (T×C) showed that combination of nanoformulated *A.indica* and *C.longa* expressed minimum fungal growth (21.400 mm) at 25ppm concentration followed by (25.133 mm) at 50ppm concentration and (29.144 mm) at 75ppm respectively. While *A.indica* NPs expressed minimum fungal growth (24.028 mm, 31.167 mm and 34.833 mm). *A.indica* (29.144 mm, 31.176 mm and 35.300 mm), *C.longa* NPs (31.367 mm, 34.500 mm and 40.078 mm) and *C.longa* (36.011 mm, 41.344 mm and 44.211 mm) at the concentrations of 25ppm, 50ppm and 75ppm respectively. The interaction of treatment and duration (T×D) exhibited that minimum fungal growth was expressed by combination of nanoformulated *A.indica* and *C.longa* (23.187 mm, 25.511 mm and 27.244 mm) followed by *A.indica* NPs (28.000 mm, 30.472 mm and 33.556 mm), *A.indica* (29.978 mm, 32.522 mm and 34.011 mm) *C.longa* NPs (33.711 mm, 35.267 mm and 37.967 mm) and *C.longa* (38.600 mm, 40.689 mm and 42.278 mm) at the interval of 24, 48 and 72h respectively.

Table 1. ANOVA for Invitro evaluation of phytoextracts against *Colletotrichum falcatum*.

Source	DF	MS	F	P
Treatment	5	5549.82	808.01	0.000*
Concentration	2	397.55	57.88	0.000*
Time	2	5549.82	808.01	0.000*
Trt * Conc.	10	129.00	18.78	0.000*
Trt *Time	10	0.53	0.08	0.000*
Conc. * Time	4	00.56	0.08	0.988 ^{NS}
Trt *Conc. *Time	20	1.04	0.15	1.000 *
Error	108	6.87		
Total	161			

*=Significant P<0.05 NS= Non significant

Scanning electron microscopy (SEM) with EDX analysis of phyto-extracts nanoparticles: Scanning electron microscope (SEM) with EDX analysis confirmed the morphology of phyto-extracts that shown in Fig 1, Fig. 2 and Fig.3.

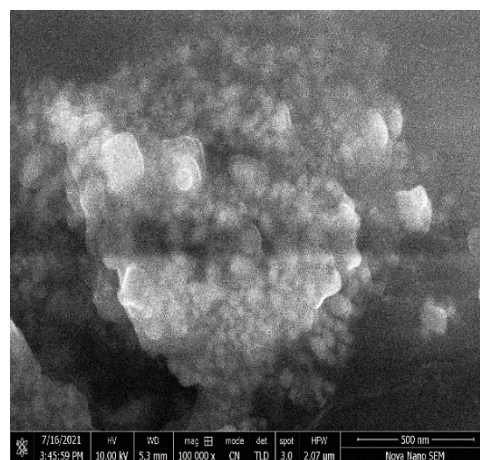


Figure 1. SEM analysis of phyto-extracts with 500nm resolution.

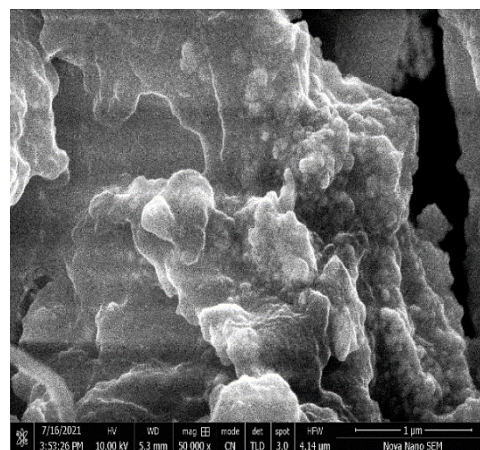


Figure 2. SEM analysis of phyto-extracts with 1 µm resolution.

Invitro evaluation of nanoformulated fungicides against red rot of sugarcane caused by *colletotrichum falcatum*: Treatments (T), Concentrations (C), Durations (D) and their interactions including treatments × concentrations (T×C) and treatments × durations (T×D) showed significant results (Table 2). Carbendazim NPs + Mancozeb NPs exhibited minimum fungal growth that was (18.244 mm) followed by Carbendazim NPs (20.474 mm), Mancozeb NPs (22.426 mm), Carbendazim (26.744 mm), Mancozeb (33.063 mm) and control expressed (55.311 mm) as compared to control.



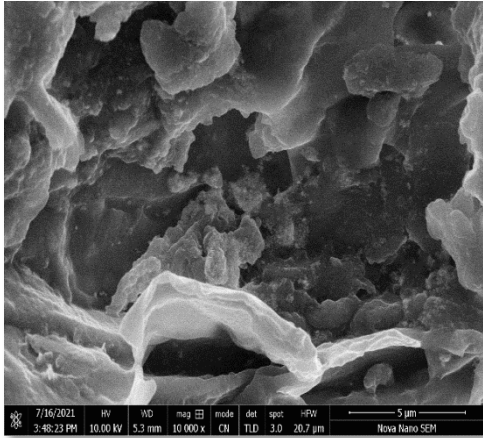


Figure 3. SEM analysis of phyto-extracts with 5 μ m resolution.

Table 2. ANOVA for Invitro evaluation of nanoformulated fungicides against *Colletotrichum falcatum*.

Source	DF	MS	F	P
Treatment	5	5311.61	2424.23	0.000*
Concentration	2	325.66	148.63	0.000*
Time	2	73.87	33.72	0.000*
Trt * Conc.	10	106.95	48.81	0.000*
Trt *Time	10	0.36	0.17	0.000*
Conc. * Time	4	0.12	0.06	0.994 ^{NS}
Trt *Conc. *Time	20	0.21	0.10	1.000*
Error	108	2.19		
Total	161			

*Significant $P < 0.05$ NS = Non significant

The interaction between treatment and concentration ($T \times C$) showed that Carbendazim NPs + Mancozeb NPs expressed minimum fungal growth (14.989 mm) at 75ppm concentration followed by 50ppm (18.189 mm), 25ppm (21.556 mm), respectively, Carbendazim NPs (16.100, 17.767, 21.544 mm), Mancozeb NPs (17.900, 22.221, 27.167 mm), Carbendazim (23.333, 26.467, 30.433 mm) and Mancozeb (29.222, 31.500, 38.467 mm) at the concentrations 25ppm, 50ppm and 75ppm respectively. Interaction of treatment and duration ($T \times D$) exhibited that minimum fungal growth was expressed by Carbendazim NPs + Mancozeb NPs (17.111, 18.278, 19.344 mm) followed by Carbendazim NPs (17.444, 18.444, 19.789 mm), Mancozeb NPs (21.111, 22.456, 23.711 mm), Carbendazim (25.991, 26.733, 27.589 mm) and Mancozeb (32.000, 33.000, 34.189 mm) after the interval of 24, 48 and 72h respectively.

Scanning electron microscopy (SEM) with EDX analysis of commercial fungicides nanoparticles: Scanning electron microscope (SEM) with EDX analysis confirmed the morphology of phyto-extracts that shown in Fig.4, Fig. 5, Fig.6 and Fig. 7.

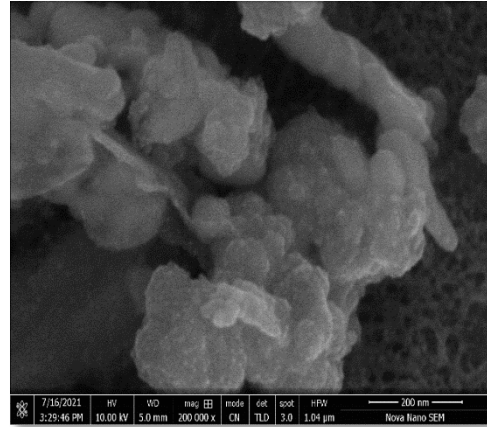


Figure 4. SEM analysis of fungicides with 200nm resolution.

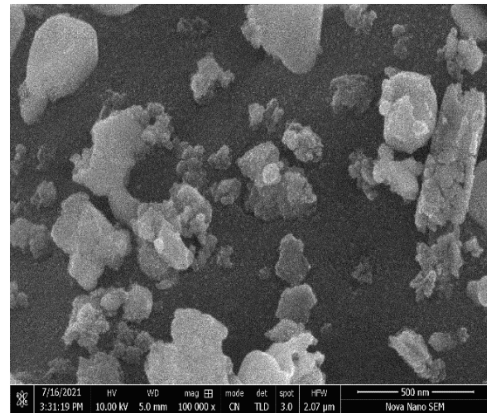


Figure 5. SEM analysis of fungicides with 500nm resolution.

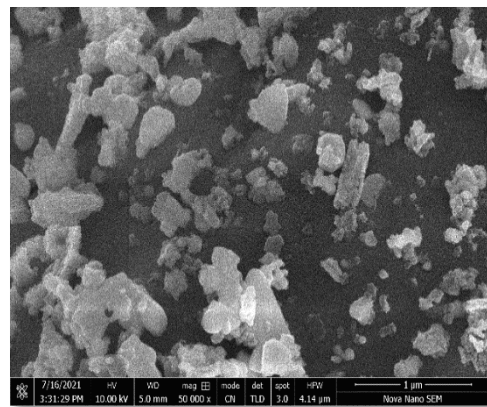


Figure 6. SEM analysis of fungicides with 1 μ m resolution.



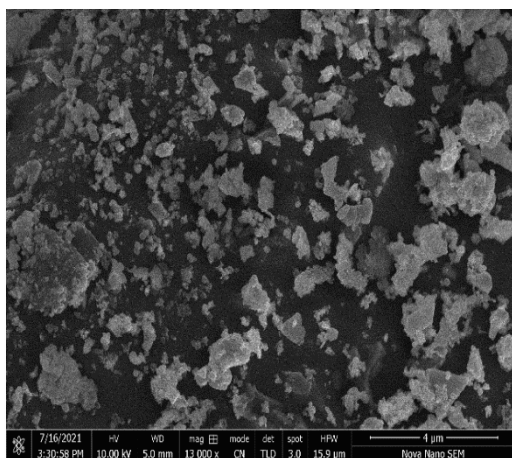


Figure 7. SEM analysis of fungicides with 4 μm resolution.

Evaluation of effective nanoformulated phtoextracts and fungicides in combinations against *Colletotrichum falcatum* under lab conditions: Treatments (T), Concentration (C), Duration (D) and there combinations including treatment × concentration (T×C) and treatment × Duration (T×D) gave significant results (Table 3). Combination of Carbendazim +AgNo3 +*A.indica* resulted in minimum fungal growth that was (16.519 mm) followed by Carbendazim +AgNo3 + *C.longa* (18.407 mm), Carbendazim +ZnO + *A.indica* (21.074 mm), Mancozeb +AgNo3 + *A.indica* (23.074 mm), *C.longa* + Mancozeb +AgNo3 (25.052 mm) and Mancozeb + ZnO + *A.indica* (27.619 mm).

Table 3. ANOVA for evaluation of the effectiveness of combination of nanoformulated phytoexytracts and fungicides against *C. falcatum* under lab conditions.

Source	DF	MS	F	P
Treatment	6	7687.99	1089.23	0.000 *
Concentration	2	723.56	102.51	0.000 *
Time	2	207.18	29.35	0.000 *
Trt * Conc.	12	131.54	18.64	0.000*
Trt *Time	12	1.58	0.22	0.000*
Conc. * Time	4	0.23	2.05	0.997 ^{NS}
Trt *Conc. *Time	32	0.97	0.14	0.967 ^{NS}
Error	126	7.06		
Total	188			

*Significant P<0.05 NS =Non significant

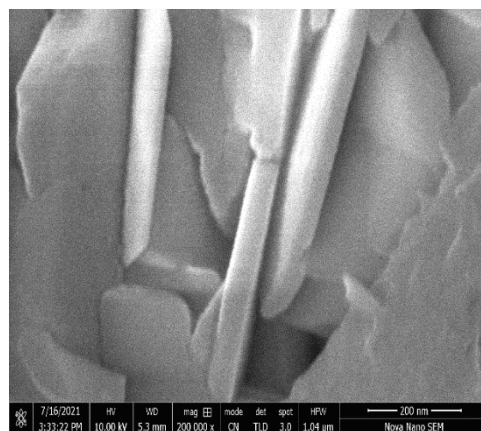


Figure 8. SEM analysis of Fungicides + phyto-extracts with 200nm resolution.

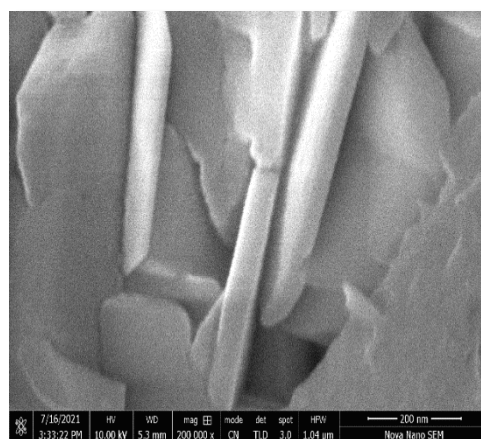


Figure 9. SEM analysis of Fungicides + phyto-extracts with 500nm resolution.

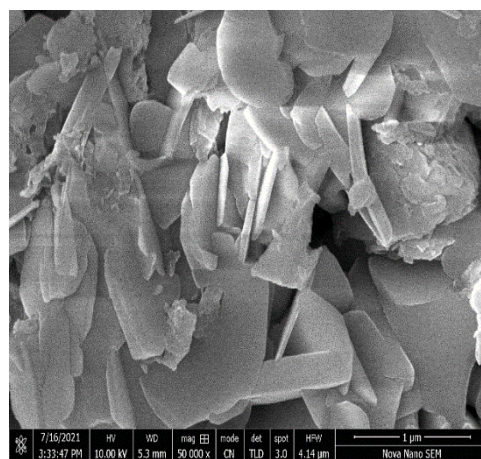


Figure 10. SEM analysis Fungicides +phyto-extracts with 1 μm resolution.



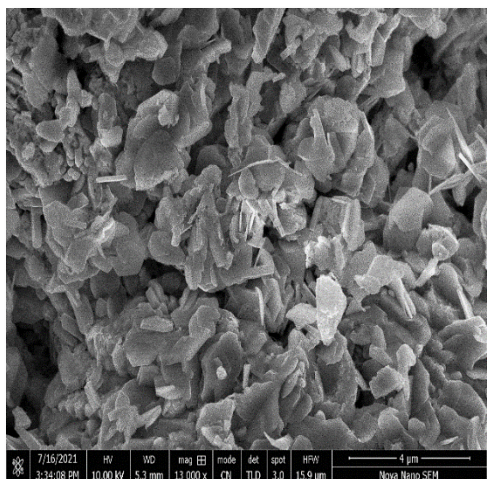


Figure 11. SEM analysis Fungicides + phyto - extracts with 4 μm resolution.

Scanning electron microscopy (SEM) with EDX analysis of combination of fungicides and phytoextracts nanoparticles: Scanning electron microscope (SEM) with EDX analysis confirmed the morphology of combination of phyto-extracts and fungicides that shown in Fig.4.8, Fig.4.9, Fig.4.10 and Fig. 4.11.

DISCUSSION

Sugarcane (*Saccharum officinarum*) is a significant cash crop grown in tropical and subtropical regions. The genus of the sugarcane is saccharum and belongs to family (*Poaceae*). It is originated in tropical Indian sub-continent and South East Asia and is broadly distributed over a large range of climatic conditions (Ram *et al.*, 2022). Brazil is the world's top producer of sugarcane, followed by the United States, India, China, Thailand, and Pakistan. With a total production of 0.083 million tons, it is grown on 1341.8 hectares in Pakistan. Punjab is the province in Pakistan where it is grown the most, followed by Sindh and KPK. It has an excessive amount of carbohydrates and is fed to both people and animals. It is an important crop for the development of bio factories since it yields high-value products like as sugar, rum, cane syrup, fibers, waxes, plastics, organic fertilizers, and biofuel. In Pakistan, there are several diseases that include sugarcane mosaic virus, whipe smut, red rot, and red stripe. Fungus causes three major sugarcane diseases that are red rot of sugarcane, whipe smut of sugarcane and Pokkah boeng. Red rot among these fungal diseases is one of the most destructive and extreme threats in many countries under favourable conditions. Under favourable conditions disease is widely spread in susceptible varieties. In Pakistan, it is mainly reported in the central and northern areas of Punjab. A reduction of around 29–83% in cane weight and 31–75% in

sugar recovery is caused by the fungal infection. Worldwide, 5–10% of losses are attributed to this disease. Certain management techniques, including as chemical, cultural, and biological control, can be used to control the disease. In the current study, two chemicals were tested in lab conditions against *C. falcatum*. The use of chemicals can have negative environmental impacts and increase the risk of pathogens developing cross-resistance to the chemical (Menossier *et al.*, 2008). That's why; phytoextracts were also evaluated against the pathogen in lab conditions. Two species of plants i.e. *Azardichta indica* (Neem) and *curcuma longa* (turmeric) were evaluated against fungus in lab conditions. Similarly, two chemicals as Carbendazim and Mancozeb were evaluated against fungus in lab conditions. The chemicals were evaluated against fungus experiments were designed under CRD. Out of the chemicals, carbendazim NPs × Mancozeb NPs were the most effective in lab conditions showed minimum fungal growth of 18.244 mm and carbendazim NPs 20.474 mm, respectively, among phyto extracts, *A.indica* NPs × *C.longa* NPs exhibited minimum fungal growth 25.311 mm respectively (Ghazanfar *et al.*, 2017). These effective chemicals and phytoextracts were also evaluated under lab conditions. These proved effective in lab condition as well. These effective chemicals and phytoextracts were also evaluated in combinations under lab conditions and Carbendazim NPs + AgNO₃ + *A.indica* showed minimum fungal growth of 16.519 mm, Whereas, Mancozeb NPs + ZnO + *A.indica* showed maximum fungal growth of 27.619mm. AgNPs and ZnNPs' monodispersed sphere morphology was confirmed by ultrastructure imaging using scanning electron microscopy (SEM) with EDX analysis.

Conclusion: The present study demonstrated that all treated nanopesticides exhibited effective control against red rot disease in sugarcane when compared to the control group. Among the various nanopesticide treatments, the combination of Carbendazim nanoparticles (NPs), Silver Nitrate (AgNO₃), and Azadirachta indica (neem) emerged as the most effective. This specific combination significantly outperformed other nanopesticide treatments in managing red rot caused by the pathogen *Colletotrichum falcatum*. The effectiveness of this treatment was evidenced by the minimal fungal growth, measured at 16.519 mm, which was substantially lower than the growth observed with other combinations. The superior performance of this particular nanopesticide blend can be attributed to the synergistic effects of its components, which likely enhance antifungal activity through multiple mechanisms of action. These findings suggest that the Carbendazim NPs + AgNO₃ + *A. indica* combination holds considerable promise for developing advanced, sustainable, and effective control strategies for red rot in sugarcane cultivation.



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Ethical statement: This article does not contain any studies with human participants or animal performed by any of the authors.

Availability of data and material: We declare that the submitted manuscript is our work, which has not been published before and is not currently being considered for publication elsewhere.

Consent to participate: Ahmad Nisar and Ghulam Mustafa Sahi conceived the idea and prepared manuscript.

Consent for publication: All authors are giving the consent to publish this research article in Phytopathogenomics and Disease Control.

REFERENCES

- Abbas, H.T., S.T. Sahi, A. Habib and S. Ahmed. 2016. Laboratory evaluation of fungicides and plant extracts against different strains of *Colletotrichum falcatum* the cause of red rot of sugarcane. Pakistan Journal of Agricultural Sciences 53:181-186.
- Agnihotri, V. 1983. Smut of Sugarcane. Diseases of Sugarcane and sugarbeet. Oxford and IBH Publishing Co 66:65-86.
- Ahmad, L., N. Pathak and R.K. Zaidi. 2016. Antifungal potential of plant extracts against seed-borne fungi isolated from barley seeds (*Hordeum vulgare* L.). Journal of Plant Pathology and Microbiology 7:5-8.
- Alexander, K. and R. Viswanathan. 2002. Diseases of Sugarcane in India and its rapid diagnosis. Sugarcane crop management 10-51.
- Anwar, M.S., H.W.A. Khan, M. Muhammad, A.A. Chattha and Z. Amjad. 2010. Integrated control strategies for Sugarcane disease, Pakistan Sugar Journal 25:7-9.
- Arshad, W.R. 2021. Assessing Current Situation And Future Prospects Of Sugarcane Ratooning Ability In Pakistan. Pakistan Sugar Journal 36.
- Bharti, Y., S. Vishwakarma, A. Kumar, A. Singh, M. Sharma and D. Shukla. 2012. Physiological and pathological aspects of some new isolates of *Colletotrichum falcatum* causing red rot disease in *Saccharum spp.* Complex. Acta Phytopathologica Entomologica Hungarica 47:35-50.
- Chand, J., J. Dang and T. Kapoor. 1974. Systemic chemicals as Sugarcane sett protectants. Science and culture 40: 69-70.
- Damasceno, C.L., J.O.D. Sá, R.M.D. Silva, C.O.D. Carmo, L.S.A.M. Haddad, A.C.F. Soares and E.A.A. Duarte. 2019. *Penicillium citrinum* as a potential biocontrol agent for sisal bole rot disease. Journal of Agricultural Science 11:206.
- Duhan, J.S., R. Kumar, N. Kumar, P. Kaur, K. Nehra, and S. Duhan. 2017. Nanotechnology: The new perspective in precision agriculture. Biotechnology Reports 15:11-23.
- Estrada-Martínez, R., E. Favela-Torres, O. Soto-Cruz and G. Saucedo-Castañeda. 2018. Use of the organic fraction of food waste for bioethanol production in a batch bioreactor. in book of proceedings 289.
- Farooq, N. and S.H. Gheewala. 2019. Water use and deprivation potential for sugarcane cultivation in Pakistan. Journal of Sustainable Energy and Environment 10:33-93.
- Ghazanfar, M.U., W. Raza and S.K. Gondal. 2017. Screening of sugarcane cultivars against *colletotrichum falcatum* causing red rot disease and its control with different fungicides under laboratory conditions. Pakistan Journal of Phytopathology 29:103-110.
- Gopal, J.V., M. Thenmozhi, K. Kannabiran, G. Rajakumar, K. Velayutham and A.A. Rahuman. 2013. Actinobacteria mediated synthesis of gold nanoparticles using *Streptomyces* sp. VITDDK3 and its antifungal activity. Materials Letters 93:360-362.
- Goyal, V., D. Rani, S. Ritika, Mehrotra, C. Deng and Y. Wang. 2023. Unlocking the Potential of Nano-Enabled Precision Agriculture for Efficient and Sustainable Farming. Plants 12:3744.
- Hassan, M.N., S. Afghan and F.Y. Hafeez. 2010. Suppression of red rot caused by *Colletotrichum falcatum* on sugarcane plants using plant growth-promoting rhizobacteria. Biocontrol 55:531-542.
- Hossain, M.I., K. Ahmad, Y. Siddiqui, N. Saad, Z. Rahman, A.O. Haruna, and S.K. Bejo. 2020. Current and prospective strategies on detecting and managing *colletotrichumfalcatum* causing red rot of sugarcane. Agronomy 10:1253.
- KHAN, F. 2015. Role of extension services on production of sugarcane in district Mardan and Charsadda: Khyber Pakhtunkhwa-Pakistan (Doctoral dissertation, the university of agriculture peshawar-pakistan).
- Khan, M.T., I.A. Khan, S. Yasmeen, G.S. Nizamani and S. Afghan. 2019. Sugarcane biofuels and bioenergy production in Pakistan: Current scenario, potential, and future avenues 175-202.
- Khan, M.A.U., M.T. Aslam, A. Rehman, M. Nawaz, M.J. Khan, M.A. Ayub, A.A. Khan, M.A. Rehman, B.A. Shahzad, S. Hussain and M.U. Hassan. 2021. Ratooning potential of different promising sugarcane clones under varying trench spacing. Journal of Innovative Sciences 7:71-77.
- Khodakovskaya, M., E. Dervishi, M. Mahmood, Y. Xu, Z. Li, F. Watanabe and A. S. Biris. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. American Chemical Society nano 3:3221-3227.



- Kumar, N., T. Jhang and T.R. Sharma. 2011. Molecular and pathological characterization of *Colletotrichum falcatum* infecting subtropical Indian Sugarcane. *Journal of Phytopathology* 159:260-267.
- Kumari, M., S.S. Khan, S. Pakrashi, A. Mukherjee and N. Chandrasekaran. 2011. Cytogenetic and genotoxic effects of zinc oxide nanoparticles on root cells of *Allium cepa*. *Journal of hazardous materials* 190:613-621.
- Lee, C.W., S. Mahendra, K. Zodrow, D. Li, Y.C. Tsai, J. Braam and P.J. Alvarez. 2010. Developmental phytotoxicity of metal oxide nanoparticles to *Arabidopsis thaliana*. *Environmental Toxicology and Chemistry: An International Journal* 29:669-675.
- Menossier, M., M. Silva-Filho, M. Vincentz, M.-A. Van-Sluis and G. Souza. 2008. Sugarcane functional genomics: Gene discovery for agronomic trait development. *International journal of plant genomics* 458732.
- Misra, V., A.K. Mall, S. Solomon and M.I. Ansari. 2022. Post-harvest biology and recent advances of storage technologies in sugarcane. *Biotechnology Reports* 33:e00705.
- Muhammad, U., T.N. Khattak, H. Rahman, M.K. Daud, W. Murad and A. Azizullah. 2018. Effects of Neem (*Azadirachta indica*) seed and Turmeric (*Curcuma longa*) rhizome extracts on aphids control, plant growth and yield in okra. *Journal of Applied Botany and Food Quality* 91:194-201.
- Naseer, M., U. Aslam, B. Khalid and B. Chen. 2020. Green route to synthesize Zinc Oxide Nanoparticles using leaf extracts of *Cassia fistula* and *Melia azadarach* and their antibacterial potential. *Scientific Reports* 10:9055.
- Pasha, A.N., U. Imtiaz, M. Zubair, M.H. Arif, R.S. Rehman, S. Muntaha, A. Amin and A. Riaz. 2023. Interspecific Hybridization as a Primary Force in Evolutionary Transformation of Fungi. *South Asian Journal of Parasitology* 6:185-200.
- Peters, R.J., H. Bouwmeester, S. Gottardo, V. Amenta, M. Arena, P. Brandhoff and K. Aschberger. 2016. Nanomaterials for products and application in agriculture, feed and food. *Trends in Food Science & Technology* 54:155-164.
- Qureshi, M. A., and Afghan, S. 2005. Sugarcane cultivation in Pakistan. Sugar Book Pub. Pakistan Society of Sugar Technologist.
- Ram, B., R. Karupaiyan and G. Hemaprabha. 2022. Sugarcane Breeding. In *Fundamentals of Field Crop Breeding* 499-570.
- Satyavir, S. 2003. Prof. Ms pavgi award lecture-red rot of Sugarcane-current scenario-Satyavir. *Indian Phytopathology* 56:245-254.
- Shang, Y., M. Hasan, G.J. Ahammed, M. Li, H. Yin and J. Zhou. 2019. Applications of nanotechnology in plant growth and crop protection: a review. *Molecules* 24:2558.
- Sharma, G., J. Singh, A. Arya and S.R. Sharma. 2017. Biology and management of sugarcane red rot: A review. *Plant Archives* 17:775-784.
- Shaukat, M. 2009. A comprehensive studies on Sugarcane. University of Agriculture, Faisalabad, Pakistan.
- Singh, N., P. Govindraj and B. Singh. 2008. Parental influence on red rot resistance in progenies of varietal crosses in Sugarcane. *Indian Sugar* 57:37.
- Thakur, A., D. Chahar and P. Thakur. 2022. Synthesis of nanomaterials by biological route. In *synthesis and applications of nanoparticles* 77-119.
- Ul Haq, I., M.K. Sarwar, A. Faraz and M.Z. Latif. 2020. Synthetic chemicals: Major component of plant disease management. *Plant disease management strategies for sustainable agriculture through traditional and modern approaches* 53-81.
- Viswanathan, R. 2021. Red rot of sugarcane (*Colletotrichum falcatum* Went). *CABI Reviews*
- Viswanathan, R. and R. Samiyappan. 2002. Induced systemic resistance by fluorescent pseudomonads against red rot disease of Sugarcane caused by *Colletotrichum falcatum*. *Crop Protection* 21:1-10.
- Viswanathan, R., A.R. Sundar, P. Malathi, P. Padmanaban. 2011. Red Rot of Sugarcane (Ed., T.R. Shanthy). Sugarcane Breeding Institute, Coimbatore.
- Wang, N., G.W. Sundin, L.D.L. Fuente, J. Cubero, S. Tatineni, M.T. Brewer, Q. Zeng, C.H. Bock, N.J. Cuniffe, C. Wang and T. Candresse. 2024. Key challenges in plant pathology in the next decade. *Phytopathology* 114: 37-842.

